APPENDIX F

ANALYTICAL PROCEDURES FOR OUTFALL MONITORING

APPENDIX F1: INDICATOR PARAMETER OVERVIEW

Ammonia

Ammonia is a good indicator of sewage, since its concentration is much higher there than in groundwater or tap water. High ammonia concentrations may also indicate liquid wastes from some industrial sites. Ammonia is relatively simple and safe to analyze. Some challenges include the tendency for ammonia to volatilize (i.e., turn into a gas and become non-conservative) and its potential generation from non-human sources, such as pets or wildlife.

Boron

Boron is an element present in the compound borax, which is often found in detergent and soap formulations. Consequently, boron is a good potential indicator for both laundry wash water and sewage. Preliminary research from Alabama supports this contention, particularly when it is combined with other detergent indicators, such as surfactants (Pitt, IDDE Project Support Material). Boron may not be a useful indicator everywhere in the country since it may be found at elevated levels in groundwater in some regions and is a common ingredient in water softeners products. Program mangers should collect data on boron concentrations in local tap water and groundwater sources to confirm whether it will be an effective indicator of illicit discharges.

Chlorine

Chlorine is used throughout the country to disinfect tap water, except where private wells provide the water supply. Chlorine concentrations in tap water tend to be significantly higher than most other discharge types. Unfortunately, chlorine is extremely volatile, and even moderate levels of organic materials can cause chlorine levels to drop below detection levels. Because chlorine is non-conservative, it is not a reliable indicator, although if very high chlorine levels are measured, it is a strong indication of a water line break, swimming pool discharge, or industrial discharge from a chlorine bleaching process.

Color

Color is a numeric computation of the color observed in a water quality sample, as measured in cobalt-platinum units (APHA, 1998). Both industrial liquid wastes and sewage tend to have elevated color values. Unfortunately, some "clean" flow types can also have high color values. Field testing by Pitt (IDDE Project Support Material) found high color values associated for all contaminated flows, but also many uncontaminated flows, which yielded numerous false positives. Overall, color may be a good first screen for problem outfalls, but needs to be supplemented by other indicator parameters.

Conductivity

Conductivity, or specific conductance, is a measure of how easily electricity can flow through a water sample. Conductivity is often strongly correlated with the total amount of dissolved material in water, known as Total Dissolved Solids. The utility of conductivity as an indicator depends on whether concentrations are elevated in "natural" or clean waters. In particular, conductivity is a poor indicator of illicit discharge in estuarine waters or in northern regions where deicing salts are used (both have high conductivity readings).

Field testing in Alabama suggests that conductivity has limited value to detect sewage or wash water (Pitt, IDDE Project Support Material). Conductivity has some

value in detecting industrial discharges that can exhibit extremely high conductivity readings. Conductivity is extremely easy to measure with field probes, so it has the potential to be a useful supplemental indicator in subwatersheds that are dominated by industrial land uses.

Detergents

Most illicit discharges have elevated concentration of detergents. Sewage and washwater discharges contain detergents used to clean clothes or dishes, whereas liquid wastes contain detergents from industrial or commercial cleansers. The nearly universal presence of detergents in illicit discharges, combined with their absence in natural waters or tap water, makes them an excellent indicator. Research has revealed three indicator parameters that measure the level of detergent or its components-- surfactants, fluorescence, and surface tension (Pitt, IDDE Project Support Material). Surfactants have been the most widely applied and transferable of the three indicators. Fluorescence and surface tension show promise, but only limited field testing has been performed on these more experimental parameters. Methods and laboratory protocols for each of the three detergent indicator parameters are reviewed in Appendix F2.

E. coli, Enterococci and Total Coliform

Each of these bacteria is found at very high concentrations in sewage compared to other flow types, and is a good indicator of sewage or septage discharges, unless pet or wildlife sources exist in the subwatershed. Overall, bacteria are good supplemental indicators and can be used to find "problem" streams or outfalls that exceed public health standards. Relatively simple analytical methods are now available to test for bacteria indicators, although they still suffer from two monitoring constraints. The first is the relatively long analysis time (18-24 hours) to get results, and the second is that the waste produced by the tests may be classified as a biohazard and require special disposal techniques.

Fluorescence

Laundry detergents are highly fluorescent because optical brighteners are added to the formula to produce "brighter whites." Optical brighteners are the reason that white clothes appear to have a bluish color when placed under a fluorescent light. Fluorescence is a very sensitive indicator of the presence of detergents in discharges, using a fluorometer to measure fluorescence at specific wavelengths of light. Since no chemicals are needed for testing, fluorometers have minimal safety and waste disposal concerns.

Some technical concerns do limit the utility of fluorescence as an indicator of illicit discharges. The concerns include the presence of fluorescence in non-illicit flow types such as irrigation water, the considerable variation of fluorescence between different detergent brands, and the lack of a readily standard or benchmark concentration for optical brighteners. For example, Pitt (IDDE Project Support Material) measured fluorescence in mg/L of Tide™ brand detergent, and found the degree of fluorescence varied regionally, temporally, and between specific detergent formulations.

Given these current limitations, fluorescence is best combined with other detergent indicators such as surfactants. Appendix F3 should be consulted for more detailed information on analytical methods and experimental field testing using fluorescence as an indicator parameter.

Fluoride

Fluoride is added to drinking water supplies in most communities to improve dental health, and normally found at a concentration of two parts per million in tapwater. Consequently, fluoride is an excellent conservative indicator of tap water discharges or leaks from water supply pipes that end up in the storm drain. Fluoride is obviously not a good indicator in communities that do not fluoridate drinking water, or where individual wells provide drinking water. One key constraint is that the reagent used in the recommended analytical method for fluoride is considered a hazardous waste, and must be disposed of properly.

Hardness

Hardness measures the positive ions dissolved in water and primarily include magnesium and calcium in natural waters, but are sometimes influenced by other metals. Field testing by Pitt (IDDE Project Support Material) suggests that hardness has limited value as an indicator parameter, except when values are extremely high or low (which may signal the presence of some liquid wastes). Hardness may be applicable in communities where hardness levels are elevated in groundwater due to karst or limestone terrain. In these regions, hardness can help distinguish natural groundwater flows present in outfalls from tap water and other flow types.

pH

Most discharge flow types are neutral, having a pH value around 7, although groundwater concentrations can be somewhat variable. pH is a reasonably good indicator for liquid wastes from industries, which can have very high or low pH (ranging from 3 to 12). The pH of residential wash water tends to be rather basic (pH of 8 or 9). The pH of a discharge is very simple to monitor in the field with low cost test strips or probes. Although pH data is often not conclusive by itself, it can identify problem outfalls that merit follow-up investigations using more effective indicators.

Potassium

Potassium is found at relatively high concentrations in sewage, and extremely high concentrations in many industrial process waters. Consequently, potassium can act as a good first screen for industrial wastes, and can also be used in combination with ammonia to distinguish wash waters from sanitary wastes. (See Chapter 12). Simple field probes can detect potassium at relatively high concentrations (5 mg/L), whereas more complex colorimetric tests are needed to detect potassium concentrations lower than 5 mg/L.

Surface Tension

Surfactants remove dirt particles by reducing the surface tension of the bubbles formed in laundry water when it is agitated. Reduced surface tension makes dirt particles less likely to settle on a solid surface (e.g., clothes or dishes) and become suspended instead on the water's surface. The visible manifestation of reduced surface tension is the formation of foam or bubbles on the water surface. Pitt (IDDE Project Support Material) tested a very simple procedure to measure surface tension that quantifies the formation of foam and bubbles in sample bottles. Initial laboratory tests suggest that surface tension is a good indicator of surfactants, but only when they are present at relatively high concentrations. Section F3 provides a more detailed description of the surface tension measurement procedure.

Surfactants

Surfactants are the active ingredient in most commercial detergents, and are typically measured as Methyl Blue Active Substances (or MBAS). They are a synthetic replacement for soap, which builds up deposits on clothing over time. Since surfactants are not found in nature, but are always present in detergents, they are excellent indicators of sewage and wash waters. The presence of surfactants in cleansers, emulsifiers and lubricants also makes them an excellent indicator of industrial or commercial liquid wastes. In fact, research by Pitt (IDDE Project Support Material) found that detergents were an excellent indicator of "contaminated" discharges in Alabama (i.e., discharges that were not tap water or groundwater). Several analytical methods are available to monitor surfactants. Unfortunately, the reagents used involve toluene, chloroform, or benzene, each of which is considered hazardous waste with a potential human health risk. The most common analysis method uses chloroform as a reagent, and is recommended because it is relatively safer when compared to other reagents.

Turbidity

Turbidity is a quantitative measure of cloudiness in water, and is normally measured with a simple field probe. While turbidity itself cannot always distinguish between contaminated flow types, it is a potentially useful screening indicator to determine if the discharge is contaminated (i.e., not composed of tap water or groundwater).

Research Indicators

In recent years, researchers have explored a series of other indicators to identify illicit discharges, including fecal steroids (such as coprostanol), caffeine, specific fragrances associated with detergents and stable isotopes of oxygen. Each of these research indicators is profiled in Pitt (IDDE Project Support Material) and summarized below in Table F1. Most research indicators require sophisticated equipment and specific expertise that limit their utility as a general indicator, given the high sampling cost and long turn-around times needed. To date, field tests of research indicators have yielded mixed results, and they are currently thought to be more appropriate for special research projects than for routine outfall testing. While they are not discussed further in this manual, future research and testing may improve their utility as indicators of illicit discharges.

Table F1: Summary of Research Indicators Used for Identifying Inappropriate Discharges into Storm Drainage		
Parameter Group	**Comments**	**Recommendation**
Coprostanol and other fecal sterol compounds	Used to indicate presence of sanitary sewage	Possibly useful. Expensive analysis with GC/MSD. Not specific to human wastes or recent contamination. Most useful when analyzing particulate fractions of wastewaters or sediments.
Specific detergent compounds (LAS, fabric whiteners, and perfumes)	Used to indicate presence of sanitary sewage	Possibly useful. Expensive analyses with HPLC. A good and sensitive confirmatory method.
Pharmaceuticals (colfibric acid, aspirin, ibuprofen, steroids, illegal drugs, etc.)	Used to indicate presence of sanitary sewage	Possibly useful. Expensive analyses with HPLC. A good and sensitive confirmatory method.
Caffeine	Used to indicate presence of sanitary sewage	Not very useful. Expensive analyses with GC/MSD. Numerous false negatives, as typical analytical methods not suitably sensitive.
DNA profiling of microorganisms	Used to identify sources of microorganisms	Likely useful, but currently requires extensive background information on likely sources in drainage. Could be very useful if method can be simplified, but with less specific results.
UV absorbance at 228 nm	Used to identify presence of sanitary sewage	Possibly useful, if UV spectrophotometer available. Simple and direct analyses. Sensitive to varying levels of sanitary sewage, but may not be useful with dilute solutions. Further testing needed to investigate sensitivity in field trials.
Stable isotopes of oxygen	Used to identify major sources of water	May be useful in area having distant domestic water sources and distant groundwater recharge areas. Expensive and time consuming procedure. Can not distinguish between wastewaters if all have common source.
GC/MSD - Gas Chromatography/Mass Selective Detector HPLC - High Performance Liquid Chromatography		

Appendix F2: "Off-the Shelf" Analytical Methodologies

F2.1 AMMONIA (0 TO 0.50 MG/L NH_3-N)

Equipment/Supplies Needed

- Hach bench top or portable spectrophotometer or colorimeter (see ordering information below)
- ammonia nitrogen reagent set for 25-mL samples
- ammonia nitrogen standard solution

Procedure

Refer to Hach method 8155 for Nitrogen, Ammonia Salicylate Method (0 to 0.50 mg/L NH_3-N) for a 25mL sample. In this method, ammonia compounds combine with chlorine to form monochloramine. Monochloramine reacts with salicylate to form 5-aminosalicylate. The 5-aminosalicylate is oxidized in the presence of sodium nitroprusside catalyst to form a blue-colored compound. The blue color is masked by the yellow color from the excess reagent present to give a final green-colored solution.

Duration of Test for Each Sample

Because of the duration of this test, samples should be run in batches of about six. From start to finish, each batch of six samples takes about 25 minutes, including the time taken to clean the sample cells and reset the instrument between each batch.

Hazardous Reagents

According to good laboratory practice, the contents of each sample cell, after the analysis, should be poured into another properly-labeled container for proper disposal.

Ease of Analysis

This procedure is time-consuming and should be performed indoors.

Ordering Information

Vendor: Hach Company
PO Box 389
Loveland, CO 80539-0389
Tel: 800-227-4224
Fax: 970-669-2932
Website: www.hach.com

[Note: The direct-Nessler method may be preferred due to its faster reaction times, but Nessler reagent is toxic and corrosive. Nessler reagent, according to its MSDS, causes severe burns, is an acute and a cumulative poison, and is a teratogen. It also contains from 5 to 10% mercuric iodide. It is now recommended that the more sensitive salicylate method because of the lower concentrations experienced in this research, and because of its lower toxicity and easier disposal requirements. The salicylate method was therefore used for this project, although prior research found it to be somewhat less satisfactory than the Nessler method.]

Equipment/Supplies Needed for Ammonia Analysis		
Item (Catalog Number)	**Quantity**	**Price**
One of the colorimeters, or spectrophotometers, listed previously will be needed. Alternatively, a dedicated colorimeter can be used, but that will only be useable for a single analyte.		
Ammonia-Nitrogen Reagent Set (25mL test) salicylate method (2243700)	1 set of 100 tests	$180.56
Ammonia cyanurate reagent powder pillows (2395566)	1 pk of 50 pillows	$ 20.20
Ammonia salicylate reagent powder pillows (2395366)	1 pk of 50 pillows	$ 25.55

F2.2 BORON (Low range 0 to 1.50 mg/L as B)

Equipment/Supplies Needed

- A Hach bench top or portable spectrophotometer or colorimeter (see ordering information below)
- Boron test kit
- 1-inch plastic sample cells (at least 2).

Procedure

Refer to Hach Azomethine-H Method 10061, which is adapted from ISO method 9390. In this procedure, Azomethine-H, a Schiff base, is formed by the condensation of an aminonaphthol with an aldehyde by the catalytic action of boron. The boron concentration in the sample is proportional to the developed color. Follow the Hach instructions that come with the reagent set for the specific procedure.

Duration of Test for Each Sample

Each batch of six samples takes approximately 20 minutes.

Hazardous Reagents

Standard laboratory practice requires that all unwanted chemicals be properly disposed.

Ease of Analysis

The procedure is a little time consuming, but several samples can be analyzed together.

Ordering information

Vendor: Hach Company
PO Box 389
Loveland, CO 80539-0389
Tel: 800-227-4224
Fax: 970-669-2932
Website: www.hach.com

Equipment/Supplies Needed for Boron Analysis		
Item (Catalog Number)	**Quantity**	**Price***
Boron Test Kit (0-1.5 mg/L) BoroTrace (Azomethine-H) Method (2666900)	1 set of 100 tests	$50.00
BoroTrace 2 reagent (2666669)	1 pk of 100 pillows	$30.00
BoroTrace 3 reagent (2666799)	1 pk of 100 pillows	$20.65
EDTA Solution 1M (2241925)	50 mL	
DR/890 portable colorimeter Programmed with 90 tests. Includes 2 sample cells, COD & TnT tube adapter, instrument, procedure manual and batteries. Portable instrument that can be used for many different analytes, but fewer than the following instruments. (48470000)[1]	1	$929.00
DR/2500 spectrophotometer includes 6 one-inch round sample cells, instrument and procedure manual, and DR/Check Absorbance Standards. Compact laboratory instrument having many capabilities. (5900000)[1]	1	$2200.00
DR/2400 portable spectrophotometer includes one-inch sample cells, instrument and procedures manuals. Portable instrument having many capabilities. (5940000)[1]	1	$1,995.00
DR/4000 V Spectrophotometer. Visible spectrum only (320 to 1100nm). Includes 1-inch matched sample cells/ AccuVacc and 16-mm vial adapters; a Single Cell Module; 1-inch and 1-cm cell adapters; dust cover; replacement lamp kit; an illustrated manual set; and a power cord. UV-Vis laboratory instrument having vast capabilities. (48100-00)[1]	1	$5500.00
[1]*Only one spectrophotometer is needed* **The per-sample expendable cost is therefore about $2.00.*		

F2.3 COLOR (0 – 100 APHA Platinum Cobalt Units)

Equipment/Supplies needed

One Hach color test kit Model CO-1 which measures color using a color disc for comparison.

Procedure

The following procedure is described in the test kit.

Low Range

1. Place the lengthwise viewing adapter in the comparator.
2. fill one sample tube to the line underlining "Cat. 1730-00" with the sample. This will be approximately 15mL. If not using 1730-00 tubes, fill to the line founds at approximately 3 inches up from the bottom of the tube.
3. Place the tube containing the water sample into the comparator in the right-hand position.
4. Fill the other sample tube with colorless water to the line underlining "Cat. 1730-00." Insert this tube in the left-side comparator opening.
5. Hold the comparator with the tube tops pointing to a window or light source at approximately a 45 degree angle (with the light coming in through the top of the tubes). View through the openings in the front of the comparator. When viewing, use care to not spill samples from unstoppered tubes.
6. Rotate the disc until a color match is obtained. The reading obtained through the scale window is the apparent color in APHA Platinum Cobalt Units.

High Range

1. If the lengthwise viewing adapter is in place, remove it.
2. Fill one of the tubes to the 5mL mark with the water sample.
3. Insert the tube in the right top opening of the comparator.
4. Fill the other tube to the 5mL mark with clear water and insert this tube into the left opening of the comparator.
5. Hold the comparator up to a light source as explained above. The reading obtained through the scale window is multiplied by 5 to obtained the apparent color.

Duration of Test for Each Sample

One minute

Hazardous Reagents

None.

Ease of Analysis

This procedure easy and fast and can be performed outside of the laboratory.

Ordering Information

Vendor: Hach Company
PO Box 389
Loveland, CO 80539-0389
Tel: 800-227-4224
Fax: 970-669-2932
Website: www.hach.com

Equipment/Supplies Needed for Color Analysis		
Item (Catalog Number)	**Quantity**	**Price**
Color Test Kit (0-100 mg/L) (223400)	one kit	$51.50

F2.4 CONDUCTIVITY

Equipment/Supplies Needed

- Cardy pocket-sized conductivity meter model B-173 made by Horiba
- Conductivity standard that comes with the meter.

Calibration

Before any measurements can be performed the instrument must first be calibrated. The meter should hold its calibration for an extended period, but it is best to check the calibration before each sample batch.

1. Press the POWER button.
2. Place a drop of the 1.41 µs/cm standard solution onto the sensor cell.
3. Press the CAL/MODE button to display the CAL mark and 1.41. Calibration is complete when the CAL mark disappears.
4. Wash the sensor with tap water, and dry with a tissue.

Measurement

1. Check first to see which mode the instrument is in by looking for the arrow pointing at the mS/cm or µS/cm.
2. Add a drop of the sample onto the sensor cell using a pipette (or the sensor may be immersed into the sample).
3. When the smiley face ☺ appears, take a reading. Be sure to note the units.

Duration of Test for Each Sample

1 minute

Hazardous Reagents

None

Ease of Analysis

Simple and fast. Can be used in the field.

Ordering Information

Vendor: Cole-Parmer Instrument Company
625 East bunker Court
Vernon Hills, IL 60061-1844
Phone: 1-800-323-4340
FAX: 847-247-2929
Website: www.coleparmer.com

Equipment/Supplies Needed for Conductivity Analysis	
Item (Catalog Number)	**Price**
Cardy pocket-sized conductivity meter and accessories (EW-05751-10)	$269.00
Replacement cardy conductivity sensor cartridge (EW-05751-52)	$ 82.00
Replacement cardy conductivity solution kit (EW-05751-70)	$ 43.00

F2.5 DETERGENTS (0-3 ppm)

Equipment/Supplies needed

- Detergents (anionic surfactants) kit from *CHEMetrics*.

Procedure

The following procedure comes with the Detergents kit. The Detergents CHEMets® test employs the methylene blue extraction method. Anionic detergents react with methylene blue to form a blue complex that is extracted into an immiscible organic solvent. The intensity of the blue color is directly related to the concentration of "methylene blue active substances (MBAS)" in the sample. Anionic detergents are one of the most prominent methylene blue active substances. Test results are expressed in mg/L linear alkylbenzene sulfonate.

1. Rinse the reaction tube with sample, and then fill it to the 5 mL mark with sample.
2. While holding the double-tipped ampoule in a vertical position, snap the upper tip using the tip-breaking tool.
3. Invert the ampoule and position the open end over the reaction tube. Snap the upper tip and allow the contents to drain into the reaction tube.
4. Cap the reaction tube and shake it vigorously for 30 seconds. Allow the tube to stand undisturbed for approximately 1 minute.
5. Make sure that the flexible tubing is firmly attached to the CHEMet ampoule tip.
6. Insert the CHEMet assembly (tubing first) into the reaction tube making sure that the end of the flexible tubing is at the bottom of the tube. Break the tip of the CHEMet ampoule by gently pressing it against the side of the reaction tube. The ampoule should draw in fluid only from the organic phase (bottom layer).
7. When filling is complete, remove the CHEMet assembly from the reaction tube.
8. Invert the ampoule several times, allowing the bubble to travel from end to end each time.
9. Using a tissue, remove the tubing from the ampoule tip. Wipe all liquid from the exterior of the ampoule, then place a small white cap firmly onto the tip of the ampoule.
10. Place the CHEMet ampoule, flat end downward into the center tube of the comparator. Direct the top of the comparator up toward a source of bright light while viewing from the bottom. Rotate the comparator until the color standard below the CHEMet ampoule shows the closest match. If the color of the CHEMet ampoule is between two color standards, a concentration estimate can be made.

Duration of Test for Each Sample

Approximately 7 minutes per sample.

Hazardous Reagents

The main components of the double-tipped ampoule are considered hazardous, and possibly carcinogenic (contains chloroform). The used ampoule should be placed back in the test kit box for later disposal at a hazardous waste facility. Use proper safety protection when performing this test: laboratory coat, gloves, and safety glasses. It is also strongly recommended that the test be performed under a laboratory fume hood. Wash hands thoroughly after handling the kit.

Ease of Analysis

This procedure may be performed outside of a standard laboratory, if well ventilated. Produces hazardous chemicals.

Ordering Information

Vendor: *CHEMetrics*, Inc
4295 Catlett Rd
Calverton, VA 20138
Phone 1-800-356-3072
FAX 1-540-788-4856
Website: www.chemetrics.com

Equipment/Supplies Needed for Detergents Analysis		
Item (Catalog Number)	**Quantity**	**Price***
Detergent kit (anionic surfactants) (K-9400)	20 tests	$63.15
Detergent kit refill (R-9400)	20 tests	$50.45
**The per-sample expendable cost is therefore $2.52.*		

F2.6 *E. COLI*

Equipment/Supplies Needed

- Colilert reagent, sterile sample bottles for 100 mL samples
- Quanti-Tray 2000
- Colilert comparator predispensed in a Quanti-Tray/2000incubator
- UV light from IDEXX.

Enumeration Procedure

1. Add contents of one Colilert snap pack to a 100 mL room temperature water sample in a sterile vessel. The standard Colilert reagent is recommended when evaluating Enterococci simultaneously so the samples are both ready to read in 24 hours. If only *E. coli* are to be evaluated, then the faster Colilert-18 reagent can be used if reading the results in 18 hours instead of 24 hours is important.
2. Cap vessel and shake until dissolved.
3. Pour sample/reagent mixture into a Quanti-Tray/2000 and seal in an IDEXX Quanti-Tray Sealer.
4. Place the sealed tray in a 35±0.5° C incubator for 24 hours.
5. Read results according to the Results Interpretation table below. Count the number of positive wells and refer to the MPN table provided with the Quanti-Trays to obtain a Most Probable Number.

Results Interpretation

Appearance	Result
Less yellow than the comparator	Negative for total coliforms and *E. coli*
Yellow equal to or greater than the comparator	Positive for total coliforms
Yellow and fluorescence equal to or greater than the comparator	Positive for *E. coli*

Duration of Test for Each Sample

Once the Quanti-Tray sealer is warm (10 min), it takes approximately 5 minutes per sample to label, seal and incubate the Quanti-Tray. After 24 hours, it takes 1-2 minutes to read the sample results under the UV lamp.

Hazardous Reagents

Used Quanti-Trays must be disposed of in a biohazard bag and handled by appropriate biohazard disposal facility, using similar practices as for alternative bacteria analysis methods.

Ease of Analysis

Not a difficult procedure to learn. Knowledge of proper handling of bacterial specimens is necessary. Cannot be performed in the field.

Ordering information

Vendor: IDEXX
1 IDEXX Drive
Westbrook, ME 04092
Phone: 1-800-321-0207
Fax: 207-856-0630
E-mail: water@idexx.com
Website: www.idexx.com/water

Equipment/Supplies Needed for *E. coli* Analysis		
Item (Catalog Number)[1]	**Quantity**	**Price***
Colilert reagent for 100mL sample (WP200)	200-pack	$1,020.00
120mL vessel with 100mL line, sodium thiosulfate & label (WV120ST-200)	200-pack	$90.00
97-well sterile Quanti-Tray/2000 trays (WQT-2K)	100-pack	$110.00
Quality control kit (E. coli, Klebsiela, Pseudomonas A). (WKT 1001)	n/a	$120.00
Colilert comparator predispensed in a Quanti-Tray/2000 (WQT2KC)	1	$6.00
Quanti-Tray Sealer (115V) with 51-well rubber insert (WQTS2X-115)	1	$3,500.00
6 watt UV lamp 110 volt (WL160)	1	$89.00
Incubator 120V, 30-65^{o}C, 14"x14"x14" (WI300)	2	$389.00
[1] See the Enterococci table above for equipment that can be shared when conducting both analyses.		
**The per-sample expendable cost (reagent, bottle, and tray) is about $6.65.*		

F2.7 ENTEROCOCCI

Equipment/Supplies Needed

- Enterolert reagent
- Sterile sample bottles for 100 mL samples
- Quanti-Tray 2000
- Incubator
- UV light from IDEXX

Enumeration Test Procedure

1. Carefully separate a Snap Pack from its strip, taking care not to accidentally open the next pack.
2. Tap the reagent snap pack to ensure that all of the Enterolert powder is in the bottom part of the pack.
3. Open the pack by snapping back the top at the score line. Caution: Do not touch the opening of the pack.
4. Add the reagent to a 100 mL water sample in a sterile bottle.
5. Aseptically cap and seal the vessel.
6. Shake to completely dissolve reagent.
7. Pour the sample/reagent mixture into a Quanti-Tray avoiding contact with the foil pull tab. Seal the tray according to Quanti-Tray instructions.
8. Incubate for 24 hours at $41^{o}\pm5^{o}$ C.
9. Read the results at 24 hours by placing a 6 watt, 365 nm wavelength UV light within five inches of the Quanti-Tray in a dark environment. Be sure the light is facing away from your eyes and toward the Quanti-Tray. Count the number of fluorescent Quanti-Tray wells. The fluorescence intensity of positive wells may vary.
10. Refer to the MPN table provided with the Quanti-Tray to determine the Most Probable Number of Enterococci in your sample.

Procedural Notes

If the sample is inadvertently incubated over 28 hours without observation, the following guidelines apply:

- Lack of fluorescence after 28 hours is a valid negative test
- Fluorescence after 28 hours is an invalid result
- Use sterile water, not buffered water for making dilutions. Enterolert is already buffered. Always add Enterolert to the proper volume of diluted sample after making dilutions.
- For comparison, a water blank can be used when interpreting results.

Duration of Test for Each Sample

Once the Quanti-Tray sealer is warm (10 min), it takes approximately 5 minutes per sample to mix, label, seal and place the Quanti-Tray in the incubator. After 24 hours, it takes 1-2 minutes to read the sample results under the UV lamp.

Hazardous Reagents

Used Quanti-Trays must be disposed of in a biohazard bag and handled by appropriate biohazard disposal facility, just like any other bacteria analysis materials.

Ease of Analysis

Not difficult procedure to learn. Knowledge of proper handling of bacterial specimens is necessary. Cannot be performed in the field.

Ordering information

Vendor: IDEXX
1 IDEXX Drive
Westbrook, ME 04092
Phone: 1-800-321-0207
Fax: 207-856-0630
E-mail: water@idexx.com
Website: www.idexx.com/water

Equipment/Supplies Needed for Enterococci Analysis		
Item (Catalog Number)	**Quantity**	**Price***
Enteroletert reagent for 100 mL samples (WENT200)	200-pack	$ 1,020.00
120 mL pre-sterilized vessel with 100 mL line, sodium thiosulfate & label (WV120ST-200)[1]	200-pack	$ 90.00
97-well sterile Quanti-Tray/2000 trays (WQT-2K) [1]	100-pack	$ 110.00
Quality control kit (E. coli, Klebsiela, Pseudomonas A). (WKT 1001)	n/a	$ 120.00
Quanti-Tray Sealer (115V) with 51-well rubber insert (WQTS2X-115) [1]	1	$ 3,500.00
6 watt UV lamp 110 volt (WL160) [2]	1	$ 89.00
Incubator 120V, 30-65^{o}C, 14"x14"x14" (WI300) [3]	2	$ 389.00

[1] Same expendable materials as for the E. coli method, additional should be ordered for each method

[2] Same as for the E. coli method and can be shared

[3] Although the same, a second incubator is needed for the E. coli method because of the different temperature settings and the normal need to evaluate Enterococci and E. coli simultaneously

** The per-sample expendable cost (reagent, bottle, and tray) is about $6.65.*

F2.8 FLUORIDE (0 TO 2.00 MG/L F^-)

Equipment/Supplies Needed

- Hach bench top or portable spectrophotometer or colorimeter (see ordering information below)
- AccuVac Vial Adaptor (for older spectrophotometers)
- SPADNS Fluoride Reagent AccuVac Ampuls.

Procedure

Refer to Hach SPADNS Method 8029 which is adapted from Standard Methods for the Examination of Water and Wastewater. This procedure involves the reaction of fluoride with a red zirconium-dye solution. The fluoride combines with part of the zirconium to form a colorless complex, thus bleaching the red color in an amount proportional to the fluoride concentration.

Duration of Test for Each Sample

Each samples takes an average of 3 minutes to test.

Hazardous Reagents

The SPANDS reagent is a hazardous solution. The used AccuVacs should be placed back in the Styrofoam shipping container for storage and then disposed properly through a hazardous waste disposal company.

Ease of Analysis

The procedure is relatively easy and fast and can be performed in the field using a portable spectrophotometer or colorimeter. However, as for all tests, it is recommended that the analyses be conducted in a laboratory, or at least in a work room having good lighting and water.

Ordering information

Vendor: Hach Company
PO Box 389
Loveland, CO 80539-0389
Tel: 800-227-4224
Fax: 970-669-2932
Website: www.hach.com

Equipment/Supplies Needed for Fluoride Analysis	
Item (Catalog Number)	**Price**
Fluoride Reagent (SPADNS) AccuVac Ampuls [1 set of 25 AccuVacs (2 needed per test)] (2506025)	$ 17.00
Adapter, AccuVac vial (needed for older spectrophotometers DR/2000 and DR/3000) (43784-00)	$ 5.40
DR/890 portable colorimeter programmed with 90 tests. Includes 2 sample cells, COD & TnT tube adapter, instrument, procedure manual and batteries. Portable instrument that can be used for many different analytes, but fewer than the following instruments. (48470000) [1]	$ 929.00
DR/2500 spectrophotometer includes 6 one-inch round sample cells, instrument and procedure manual, and DR/Check Absorbance Standards. Compact laboratory instrument having many capabilities. (5900000) [1]	$ 2,200.00
DR/2400 portable spectrophotometer includes one-inch sample cells, instrument and procedures manuals. Portable instrument having many capabilities. (5940000) [1]	$ 1,995.00
DR/4000 V Spectrophotometer. Visible spectrum only (320 to 1100nm). Includes 1-inch matched sample cells/ AccuVacc and 16-mm vial adapters; a Single Cell Module; 1-inch and 1-cm cell adapters; dust cover; replacement lamp kit; an illustrated manual set; and a power cord. UV-Vis laboratory instrument having vast capabilities. (48100-00) [1]	$ 5,500.00
[1] only one spectrophotometer is needed **The per-sample expendable cost is about $1.36.*	

F2.9 pH

Equipment/Supplies Needed

- Cardy pocket-sized pH meter model B-213 made by Horiba
- pH standards that come with the meter.

Calibration

The meter should hold its calibration for an extended period, but it is best to check the calibration before each sample batch.

1. Press the ON/OFF button.
2. Place approximately 1 mL of the yellow pH 7.0 standard solution onto the sensor cell (be careful not to touch the sensor with the dropper or pipette, the cell is covered with a very thin and fragile glass cover slip).
3. Press the CAL button to display the black CAL mark in the upper right corner and 7.0.
4. Calibration is complete when the CAL mark disappears. Wash the sensor with tap or distilled water and dry with a tissue.
5. Press CAL again so that 4.01 and CAL are displayed to calibrate using the pink pH 4.01 buffer. Follow the same procedure as above.

Measurement

1. Place a drop of the sample water onto the sensor cell (usually around 1 mL). Alternatively, you may dip the meter into the water to be tested.
2. When the smiley face ☺ appears, read the number.
3. Press the ON/OFF button to turn the power OFF.
4. Wash the sensor with tap water or distilled water. Wipe off any residual water on the sensor with a tissue.
5. Be sure the protective cap is covering the sensor and put the pH meter back in its protective case.

Duration of Test for Each Sample

Calibration takes around 3 minutes, and testing of each sample is only about 30 seconds.

Hazardous Reagents

None

Ease of Analysis

Simple and fast. Can be used in the field.

Ordering Information

Vendor: Cole-Parmer Instrument Co.
625 East Bunker Court
Vernon Hills, IL 60061-1844
Phone: 1-800-323-4340
FAX: 847-247-2929
Website: www.coleparmer.com

Equipment/Supplies Needed for pH Analysis	
Item (Catalog Number)	**Price**
Cardy twin pH meter and accessories (EW-05759-00)	$238.00
Replacement pH sensor cartridge (EW-05759-0)	$105.00
Replacement pH solution kit (EW-05751-70)	$ 29.00

F2.10 POTASSIUM

Equipment/Supplies Needed

- Cardy potassium compact meter by Horiba model C-131
- Accessories that come with the meter.

Two-Point Calibration (Monthly)

1. Turn the power ON
2. Open the sensor cover and wipe the sensor pad clean with a piece of tissue and deionized water, then wipe it dry with a piece of tissue. Repeat this several times.
3. Place a piece of sampling sheet onto the sensor pad, and drip 2 to 5 drops of the standard STD solution onto it (or drip the solution directly onto the sensor pad).
4. After the readout has stabilized, adjust the STD dial so that the display reads 20X100. After cleaning the sensor according to step (2), follow the same procedure using the standards SLOPE solution and after the readout has stabilized, adjust slope volume so that the display reads 15X10.
5. After cleaning several times with deionized water, measure the standard STD solution again.
6. Recalibrate if the reading is not (20±2)X100.
7. Wipe the sensor pad with deionized water, then wipe it dry.

One-Point Calibration (Daily)

1. Turn the power ON.
2. Open the sensor cover, and wipe the sensor pad clean with deionized water, then wipe it dry.
3. Repeat this procedure several times.
4. Place a piece of sampling sheet onto the sensor pad, and drip 2 to 5 drops of the standard STD solution on it (or drip the solution directly onto the sensor pad).
5. After the readout has stabilized, adjust the STD dial so that the display reads 20X100.
6. Wipe the sensor pad with deionized water, and then wipe it dry.
7. If the sample is below 500 ppm (mg/L), use the SLOPE solution and adjust the STD dial to read 15X10.

Measurement

1. Place the sample directly onto the sensor pad or measurement can be aided by placing the sample onto a piece of sampling sheet.
2. Read the concentration directly from the display.
3. Clean the sensor with deionized water and wipe it clean after each sample is analyzed.
4. When finished with all samples, turn the power OFF.
5. Clean the surface of the sensor pad with deionized water and wipe dry for storage.

Duration of Test for Each Sample

Calibration takes around 5 minutes and testing of each sample is only 30 seconds.

Hazardous Reagents

None

Ease of Analysis

Simple and fast. Can be used in the field.

Ordering information

Vendor: Cole-Parmer Instrument Company
625 East Bunker Court
Vernon Hills, IL 60061-1844
Phone: 1-800-323-4340
FAX: 847-247-2929
Website: www.coleparmer.com

Equipment/Supplies Needed for pH Analysis	
Item (Catalog Number)	**Price**
Cardy potassium compact meter and accessories (EW-05755-00)	$239.00
Replacement cardy potassium sensor cartridge (EW-05755-500)	$ 64.00
Replacement cardy potassium solution kit (EW-05755-60)	$ 33.00

Note: This procedure is rapid and inexpensive, however, it only has a detection limit of about 1 mg/L, and reads in increments of 1 mg/L. This level of precision is not typically a problem for moderately contaminated samples (when the results are most useful); however, it presents challenges when used for cleaner water. Specifically, since the Flow Chart Method relies on the ammonia to potassium ratio to distinguish between washwaters and sanitary wastewaters, a "non detect" (i.e., <1) potassium concentration results in an indeterminant ratio value. Where clean water is being analyzed and more sensitive potassium values are needed, the only real option is to use other laboratory methods (either ICP or atomic absorption). Other simple field procedures (such as the method supplied by HACH) rely on a photometric measurement of a floc and are not very repeatable for these types of samples.

F2.11 TOTAL HARDNESS (10 – 4000 mg/L as CaCO3)

Equipment/Supplies Needed

- Hach digital titrator
- Total hardness titration cartridge
- ManVer 2 hardness indicator
- Hardness 1 buffer solution.

Procedure

Refer to Hach Method 8213 for Hardness, Total (10-4000 mg/L as $CaCO_3$) digital titrator method using EDTA. This procedure involves buffering the sample first to pH 10.1, adding of the ManVer 2 Hardness Indicator, which forms a red complex with a portion of the calcium and magnesium in the sample, and then titrating with EDTA. The EDTA titrant reacts first with the free calcium and magnesium ions, then with those bound to the indicator, causing it to change to a blue color at the end point.

Duration of Test for Each Sample

Approximately 5 minutes.

Hazardous Reagents

The mixture of sample, buffer solution, hardness indicator, and EDTA must be stored properly in a labeled container until disposal by a hazardous waste disposal facility.

Ease of Analysis

This procedure is not recommended to be performed in the field. Produces hazardous chemicals.

Ordering information

Vendor: Hach Company
PO Box 389
Loveland, CO 80539-0389
Tel: 800-227-4224
Fax: 970-669-2932
Website: www.hach.com

Equipment/Supplies Needed for Total Hardness Analysis

Item (Catalog Number)	Quantity	Price*
Digital Titrator with plastic case, manual and 5 straight delivery tubes (1690001)	1 titrator	$105.00
Total hardness titration cartridge (EDTA 0.0800M) (1436401)	1	$10.70
Total hardness titration cartridge (EDTA 0.800M) (1439901)	1	$10.70
Delivery tube, (straight with J hook) for titration (1720500)	Pack of 5	$4.85
ManVer 2 Hardness Indicator Powder Pillow (85199)	1 pack of 100 pillows	$9.85
Hardness 1 buffer solution (42432)	One 100 mL bottle	$8.40
**The per sample expendable cost is about $0.25, depending on the hardness level.*		

F2.12 TURBIDITY

Equipment/Supplies Needed

- Benchtop or portable turbidimeter. The range of readings in NTU will depend upon the instrument.

Procedure

(This is a general procedure for turbidity. Follow your turbidimeter's instructions):

1. First, the instrument must be calibrated using the standards supplied with the instrument. If calibration is satisfactory, continue with sample measurement.
2. Samples are normally stored under refrigeration. Before analyzing for turbidity, the samples must first be brought back to room temperature. This is done to prevent the formation of frost on the outside of the glass sample cells used in the turbidity measurement.
3. Pour the sample into a sample cell (until almost full or to the fill line), cap the cell, then turn it upside down 2 to 3 times for mixing. Do not shake vigorously.
4. Keep the sample cell vertical for 4-5 seconds and wipe the outside to remove fingerprints.
5. Place the cell into the turbidity meter and take a reading.

Duration of test for each sample

Approximately one minute. This does not include the time spent bringing the sample to room temperature.

Hazardous Reagent

None

Ease of Analysis

Relatively simple and may be performed outside of the laboratory using a portable turbidimeter.

Ordering Information

Vendor: Hach Company
PO Box 389
Loveland, CO 80539-0389
Tel: 800-227-4224
Fax: 970-669-2932
Website: www.hach.com

Equipment/Supplies Needed for Turbidity Analysis		
Item (Catalog Number)	**Quantity**	**Price**
2100P Portable Turbidimeter range 1-1000 NTU Includes nine sample cells, primary standards, silicone oil & oiling cloth, manual, quick reference card and case. (4650000)	1	$837.00

Appendix F3. METHODOLOGIES AND LAB TESTING OF TECHNIQUES TO MEASURE DETERGENTS

F3.1 *CHEMETRICS* DETERGENT TEST KIT

Detergents were measured using the *CHEMetrics* detergent test kit, which detects Methylene Blue Active Substances (MBAS), an important ingredient of detergent products. The minimum detection limit (MDL) of the kit is 0.25mg/L. This is a very simple test, but the accuracy of the tests depends on the analyst's skill with the color comparator. One of the problems with this method is the upper limit of 3 mg/L. Higher values can only be measured with dilution of the sample prior to analysis. This extra step requires extra time when measuring laundry, carwash and sewage samples, when the detergent values are in hundreds of mg/L.

This kit also contains chloroform, an expected carcinogen. Great care must therefore be taken when conducting this analysis and when handling the kit materials. The alternative detergent field test kit from HACH uses much larger quantities of benzene, also a known carcinogen, and is not as well contained as the chloroform in this preferred kit. An important aspect of this research was investigating alternative analytes that could be used instead of detergents.

The main components of the *CHEMetrics* detergent test kit (Figure F3.1) are:

1. Test tube
2. Comparator device
3. Snapper
4. Double tipped ampoule containing chloroform and other reagents (blue stained)
5. CHEMets ampoule (empty vacuum ampoule)

Figure F3.1: *CHEMetrics* detergent test kit components

Test Procedure Summary

This test should preferably be conducted in a laboratory fume hood due to the possibility of exposure to chloroform.

1. Pour 5 mL of the sample into the test tube.
2. Snap one tip of the double tipped ampoule, keeping the other tip inside the tube, but above the sample level. Invert the snapped tip into the tube and snap the other tip of the ampoule. Let the blue chemical (containing chloroform) completely empty into the test tube.
3. Cap the tube tightly and shake the solution for 30 seconds. Keep the solution undisturbed for 1 minute in a test tube rack.
4. Remove the cap from the tube and insert the vacuum CHEMets ampoule into the test tube. Care must be taken so that the small plastic tube at the tip of the ampoule touches the bottom of the tube.
5. Snap the CHEMets ampoule tip by the side of the test tube and let the solution flow through the tube into the CHEMets ampoule.
6. Take off the plastic tube and wipe off the tip of the ampoule. Put the provided white cap on the tip of the ampoule and place it in the color comparator.
7. Compare the color of the solution inside the ampoule with the color comparator. The colors range from light blue (0.25 mg/L) to dark blue (3 mg/L). If the color is darker than the given colors in the comparator, the sample needs to be diluted and retested. No color indicates <0.25 mg/L value for detergents. The test tube needs to be disposed of carefully because it contains a hazardous chemical (chloroform).

Harmful Chemicals in *CHEMetrics* Detergent Test Kit

The main components of the double tipped ampoule are methylene blue, sulfuric acid, sodium phosphate, water and chloroform. Chloroform may affect the liver, kidney and central nervous system, and is a known carcinogen. On exposure, it causes irritation to eyes, skin and mucous membranes. It may also cause burning of the throat, mouth esophagus and stomach. It may also cause nausea, vomiting and diarrhea. Wash your hands thoroughly after handling the kit and conduct the analysis in a well-ventilated area, preferably in a laboratory fume hood. Avoid contact with the eyes. Safety glasses and gloves are required while doing this test. If there is a spill, take up with an absorbent material. Keep the reagents in the ampoule for final disposal, in accordance with regulations.

F3.2 Fluorescence Monitoring using the GFL-1 Fluorometer

Introduction

Fluorescence is the property of the whiteners in detergents that cause treated fabrics to fluoresce in the presence of ultraviolet rays, giving laundered materials an impression of extra cleanliness. These are also referred to as bluing, brighteners or optical brighteners and have been an important ingredient of most laundry detergents for many years. The effectiveness of the brighteners varies by the concentration of the detergents in the wash water. The detection of optical brighteners has been used as an indicator for the presence of laundry wastewater, and municipal sewage, in urban waters.

One method of quantifying fluorescence in the laboratory is by using a fluorometer calibrated for detergents. In our tests, we used the GFL-1 Portable Field fluorometer (Figure F3.2).

The components of the GFL-1 Fluorometer are the power switch, sample chamber, battery compartment, source module, detector filter cartridge, display, keypad, and the interface port. A 1.2 Ah rechargeable lead-acid battery powers the unit when in the field. The fluorometer contains high efficiency interference filters optimized for fluorescence detection. It contains a silicon photodiode detector and a LED source. The interface port is also used as the battery charger port. A 192 X 192 dot LCD screen is used for text and graphical data presentation.

Figure F3.2: GFL-1 Portable Field Fluorometer

Calibration

Before the instrument is used, it should be calibrated with a detergent solution. No general standard detergent solution is available, so a commercially available detergent is used to prepare standard solutions. For this research, a common commercial detergent, Procter & Gamble's *Tide ™* was used. The purpose of calibrating the fluorometer is to set the instrument fluorescent signal levels to correspond to different concentrations of this commercial detergent. Single point and multipoint calibrations are available with this fluorometer. The manufacturers report that the solution used in calibration is unimportant in that the procedure is the same regardless of the solution used. A five-point calibration method is used for instrument calibration. To test a sample, the instrument must be in "test mode." The test mode cannot be used until a calibration table has been built, or an existing one is made active. If there is no active calibration table, the test mode screen will automatically default to the "calibration menu" screen.

To install a new calibration table, select CREATE CAL TABLE by pressing 1 on the keypad. Soon the cal table builder screen appears on the display. Since a five point calibration is being done, six different concentrations of Tide detergent were made: 0.5mg/L, 5mg/L, 10mg/L, 50mg/L, 100mg/L, 500mg/L. A concentration of 25 mg/L of Tide corresponds to a typical working solution for a batch of laundry. The sample bottles for the GFL-1 fluorometer come with the instrument. These are the only sample bottles that can be used for the measurement of fluorescence. There are five steps in making a calibration table:

Step 1

The screen will prompt to insert the most concentrated reference in order to set the detector gain. In this case, the highest concentration is 500mg/L. Press ENTER.

Step 2

Insert the blank and press ENTER.

Step 3

The next step is to enter the calibration units (e.g., mg/L). Pressing the ENTER key takes takes the user to the next step.

Step 4

This step prompts the user to insert a reference sample of any concentration. After inserting the reference sample, press ENTER. The screen will then prompt the user to enter the concentration value for the inserted reference sample. After setting the known reference, the screen will ask whether or not to do another point. Press YES and repeat the above sequence until you have inserted all the prepared reference samples. The reference samples should be inserted in a random fashion and not in the order of increasing or decreasing values of concentration.

Step 5

The last step prompts the user to name the calibration table. It should be noted that calibration tables are not saved until a name is given to the table. Then press ENTER.

Now the fluorometer is ready to start running samples.

Sample Test Mode

Figure F3.3 is the first screen display shown after switching on the fluorometer. Press 1 for the test mode, since the calibration table has already been saved.

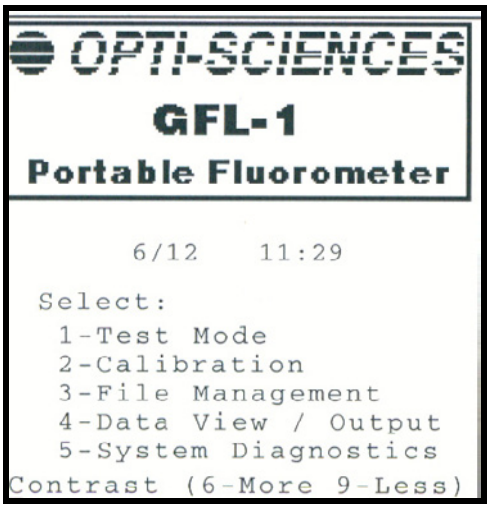

Figure F3.3: Main Menu

The screen will then display the following (Figure F3.4):

Figure F3.4: Calibration Menu

Press 2 for using the saved calibration table as the active calibration table in the memory. The next screen would prompt you to enter the desired table number saved. If you have saved only one calibration table, press 1.

Place a blank sample in the sample chamber and press ENTER (Figure F3.5). You will then see the screen displayed in Figure F3.6.

Figure F3.5: Placing Sample into Sample Chamber

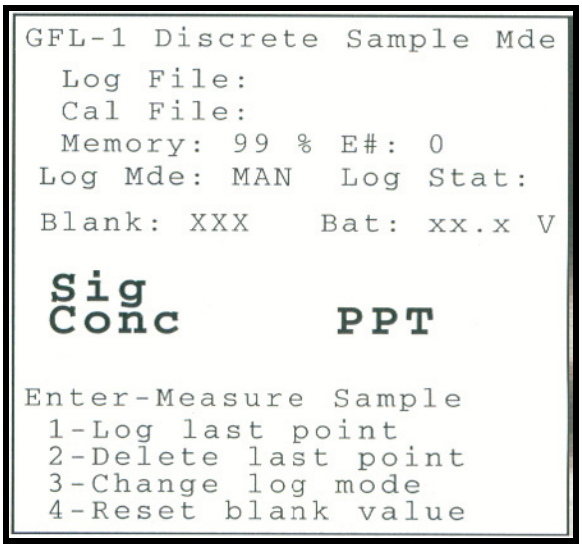

Figure F3.7: Discrete Sample Mode

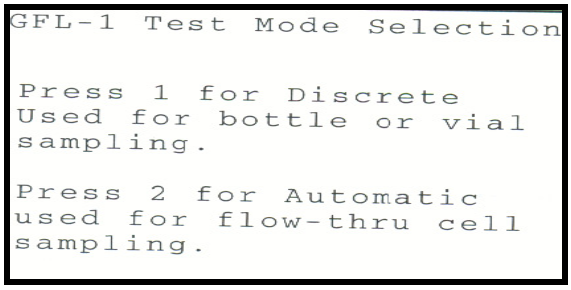

Figure F3.6: Test Mode Selection

Press 1 for doing discrete bottle sampling.
A new screen will appear (Figure F3.7).

With calibration complete, the instrument is ready to analyze the samples. To run a test, simply load a sample into the chamber and press ENTER. The unit will measure the sample and present the data a few seconds later. A busy message indicates that the test is in progress. Press ESC to return to the main menu.

Initial Tests using the Fluorometer

Initial tests were conducted after the first calibration to get an indication of the repeatability and drift of the results obtained from the new instrument. Five different concentrations of Tide detergent samples were made and tested for fluorescence after varying periods of time. The results of these tests are shown in Figure F3.8.

It is obvious that the fluorescence signal from Tide degrades with time and that the analyses should be evaluated within two hours. Other samples of commercial and household detergents were also evaluated and degradation of fluorescence with time was also identified. The largest changes occurred between about one and two hours after sample preparation. There was very little change after this initial two hour period. In the real world, the time between mixing of a laundry detergent with the washwater at the laundry, its discharge, and its analysis in the laboratory is at least two hours. Therefore, the fluorescence values used are those obtained after the signals have reached a relatively constant value. The results of the tests on certain commercial and household detergents are shown in Figure F3.9.

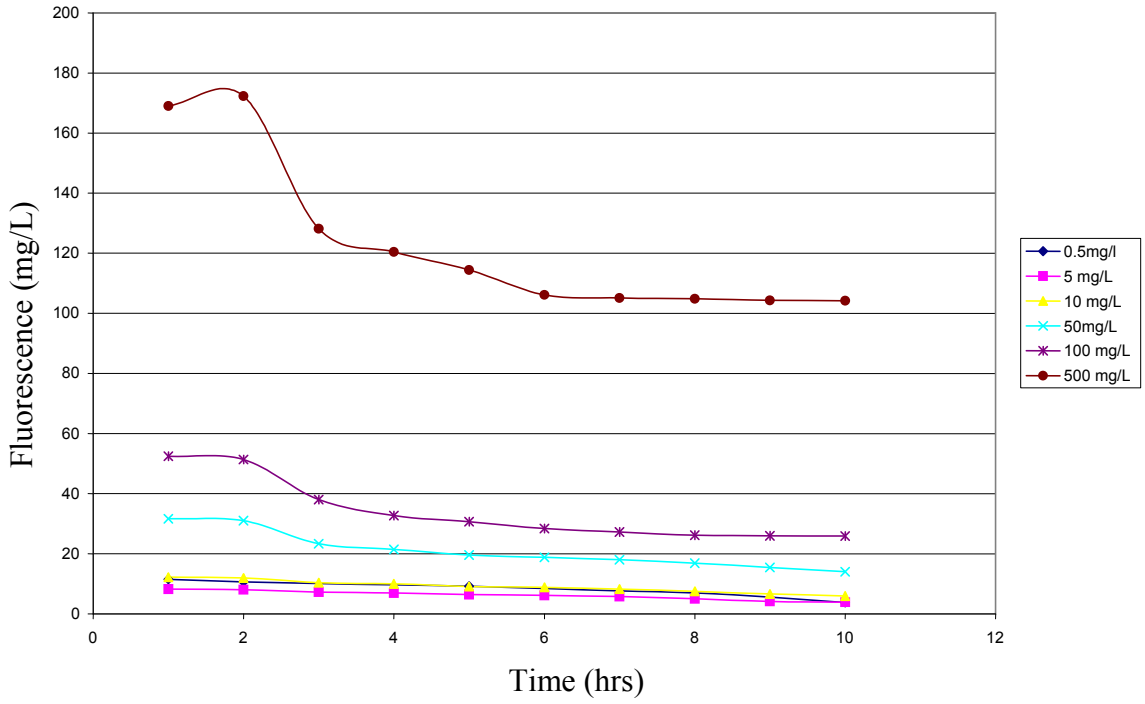

Figure F3.8: Changes in Tide DetergentFluorescence over Time

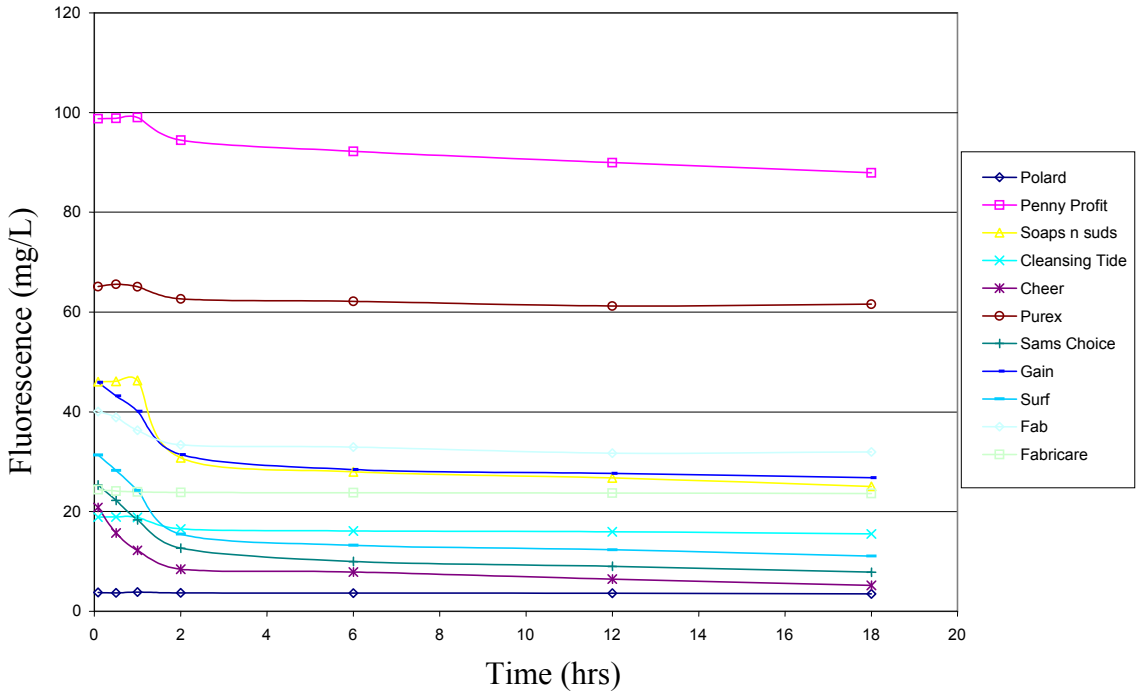

Figure F3.9: Changing Fluorescence with Time

The commercial laundry detergent samples in this graph were *Polard, Penny Profit, Soaps n Suds, and Cleansing Tide*. The others are household detergents (*Cheer, Purex, Sam's Choice, Gain, Surf, Fab, and Fabricare*). *Soaps n Suds* had a steep drop in fluorescence after one hour of preparation of the sample. After two hours, the fluorescence values stayed relatively constant without further changes. There was only one sample (*Polard*, a commercial detergent) that did not show any change in its fluorescence value. This detergent also had the lowest fluorescence signal of any of the samples. Although equal concentrations of all of these detergents were evaluated (50 mg/L), the fluorescence values ranged from 5 mg/L to 100 mg/L, as Tide. Obviously, the ingredients of the different detergents varied greatly.

F3.3 Surface Tension Test for the Detection of Detergents

Introduction

This discussion presents a proposed sensitive method to detect detergents without hazardous chemicals and with standard laboratory equipment. The method uses the property of the detergent to decrease the surface tension of the bubbles formed when the sample is agitated. Different detergents at different pHs were used during these tests. Results indicate that the method can be used to detect detergent concentrations above 1 mg/L, and can be used as a presence/absence test for concentrations above 0.3 mg/L. The method also was verified with samples collected from a known inappropriate detergent discharge.

One of the effects of detergents in water is the reduction in surface tension. When a sample of water with detergent is agitated, air is mixed with water, creating bubbles. Because the surface tension is reduced, the tension that controls the pressure of the air is low and the surface film is not destroyed. This property can be used to estimate the detergent concentration based on the amount of foam produced after the sample is agitated.

The amount of foam formed after a sample of water with detergent is agitated can be affected by various parameters. Temperature can affect the surface tension of the water. An increase in the temperature will reduce the surface tension. Foam production can also be affected by the chemical composition of the water. As an example, low pH will decrease the foam production.

The following discussion presents an inexpensive, safe, and reasonably sensitive method to estimate the detergent concentrations in a water sample using common laboratory equipment and without hazardous reagents.

Methods

General laboratory equipment was used to generate foam from samples of distilled water and detergent at different concentrations. The idea of the experiment was to drop the sample inside a burette from a constant elevation and to measure the height of the foam created 10 seconds and 1 minute after the last drop fell.

Apparatus:

- A rectangular base support and rod assembly
- A 50 mL burette
- A clamp to hold the burette
- A 25 mL blowout pipette
- Two 10 mL pipettes
- A stop watch
- A 200 mL volumetric flask
- A portable pH meter

A rectangular base support was used to hold the burette vertically. Using a 25 mL pipette, a 25 mL sample was released into the 50 mL burette. The sample was released by free fall from near the top of the burette, taking care that the sample does not touch the wall of the burette to maximize the amount of bubbles that can be produced. An initial reading of the foam height was taken 10 seconds after the pipette was drained. A final reading was obtained 50 seconds later.

Reagents:

- Detergent (Tide)
- Distilled water
- 500 mL NaOH 1N
- 500 mL H_2SO_4 0.02N

Four samples at the same concentration were created at the same time. Four stands and four burettes were used for each concentration. After the reading, the burettes were washed for more than 2 minutes until they were clean.

To obtain more foam during the experiment, the pH was increased up to 12. The sample was diluted with distilled water and 10 mL of 1N NaOH added. The sample was prepared in a 200 mL volumetric flask. NaOH was selected because it is present in most of the detergents. After the reading was taken, the sample (200 mL) was neutralized with 100 mL 0.05N H_2SO_4 before disposal.

Results

Table F3.11 shows the foam reading above the water surface 10 seconds and 1 minute after the last drop.

The results indicate that this method can be used as a presence/absence test for detergent concentrations between 0.2 and 1 mg/L (as Tide) and to estimate concentrations above 1 mg/L. The method is simple and does not require specialized equipment.

An advantage of this method is that the equipment is easily available and inexpensive. The disadvantages are the variability in readings due to changes in temperature and characteristics of the detergents.

Figure F3.10 shows the results from concentrations between 10 and 50 mg/L. For readings above 10 mg/L, if the level of detergent increases the height of the foam also increases in a parabolic shape. It was also observed that the repeatability of the results decrease at high levels.

For levels of detergent lower than 10 mg/L, there is not an important change in the reading. The minimum reading that can be obtained from the burette is 0.05 mL. For samples in this range the reading is close to the precision of the instrument. Figure F3.11 shows the results from concentrations between 0 and 5 mg/L.

Readings below 1.0 mg/L create a circle of bubbles around the wall of the pipette. This circle was not present when distilled water was used. This procedure can be used as a presence/absence test. The circle was observed for concentration of detergent higher than 0.2 mg/L.

Conclusions

The new method is an inexpensive, safe and moderately accurate method to estimate the presence of detergents in concentrations above 0.2 mg/L. For detergent concentrations above 10 mg/L, the method can be used to quantify the concentrations. These higher concentrations have been observed in sewage, industrial discharges, laundries and car wash areas.

Table F3.11: Foam Readings Over Time

Concentration (mg/L, as Tide)	Foam Height after 10 sec. (mL)	Foam Height after 1 min. (mL)
0	0	0
0.1	0	0
0.2	T	T
0.3	T	T
0.4	T	T
0.5	T	T
0.7	T	T
1	0.05, 0.05, 0.05, 0.05	0.05, 0.05, 0.05, 0.05
2	0.1, 0.1, 0.1, 0.1	0.1, 0.1, 0.1, 0.1
3	0.1, 0.1, 0.15, 0.15	0.1, 0.1, 0.15, 0.15
5	0.15, 0.15, 0.15, 0.15	0.15, 0.15, 0.15, 0.15
10	0.2, 0.2, 0.2, 0.2	0.35, 0.4, 0.4, 0.4
20	0.8, 0.6, 0.6, 0.6	1.5, 1.3, 1.4, 1.3
50	2.6, 2.6, 3.0, 2.8	3.8, 3.5, 3.7, 3.6

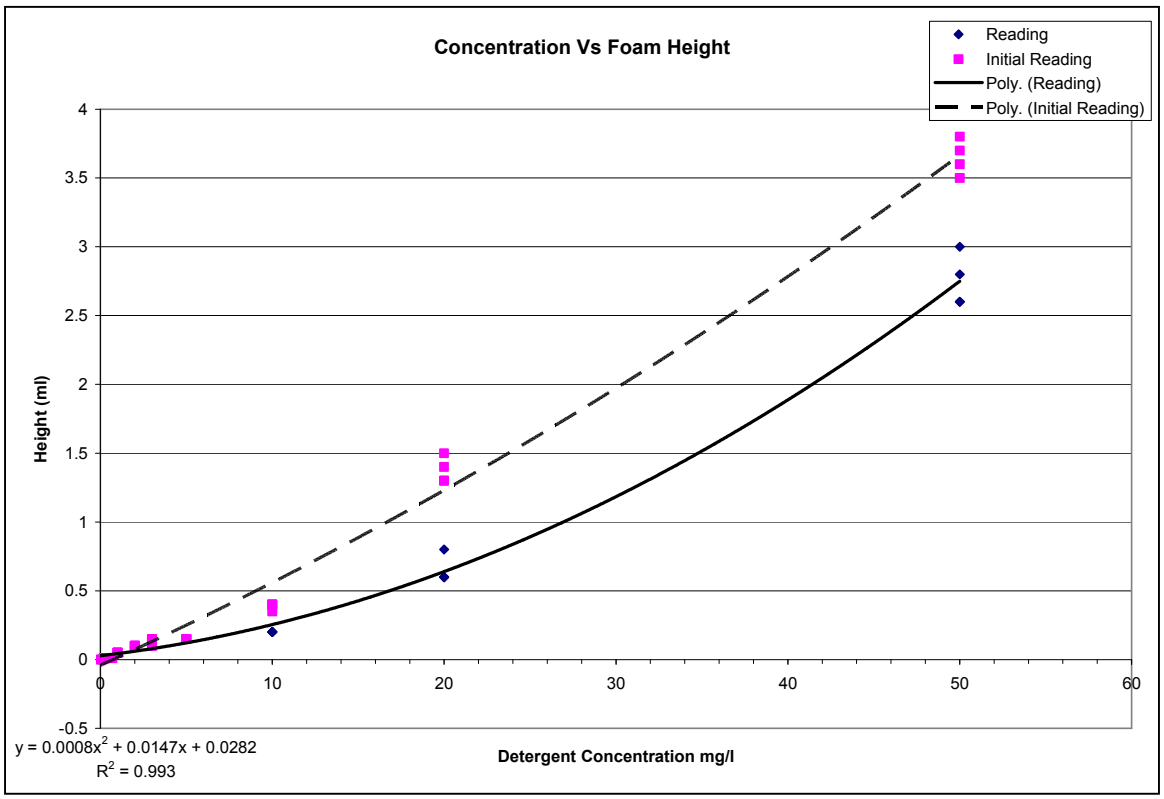

Figure F3.10: Correlation Between Concentration and Foam Height at Higher Concentrations

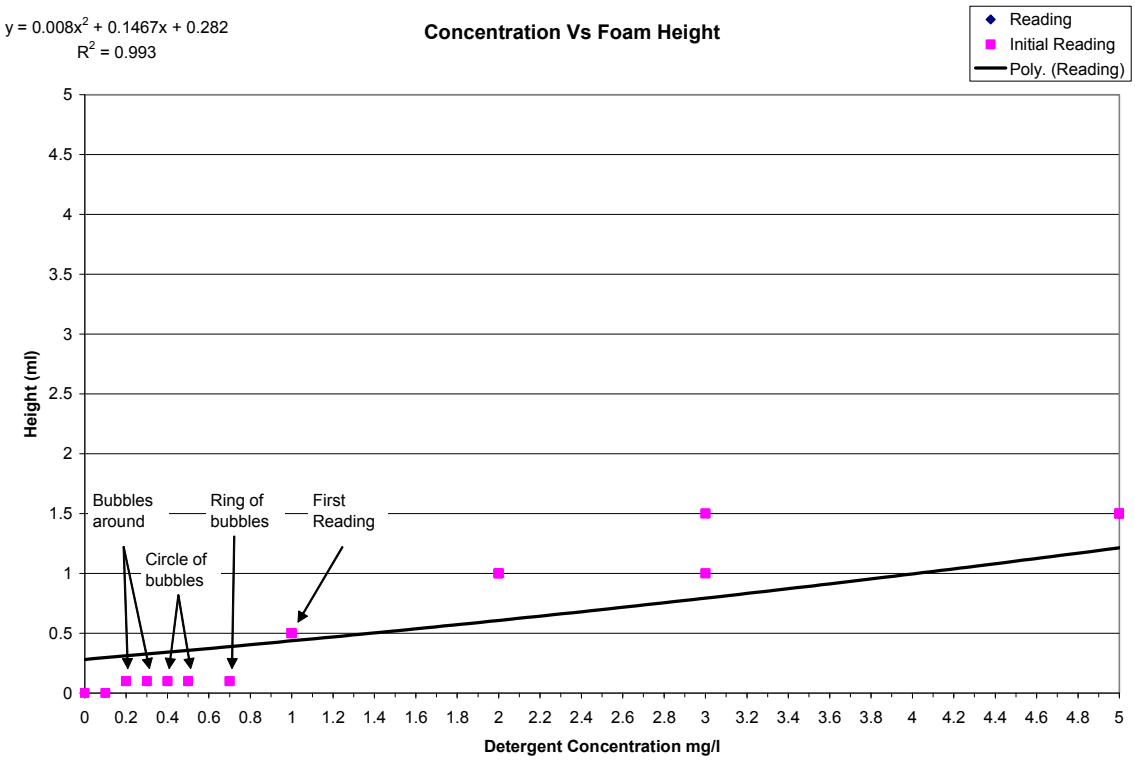

Figure F3.11: Correlation Between Concentration and Foam Height at Lower Concentrations

APPENDIX F4: LAB TESTING OF "OPTICAL BRIGHTENER MONITORING" TO FIND INTERMITTENT DISCHARGES

Introduction

Fabric brighteners are fluorescent dyes added to soaps and detergents. These are used to produce a brightening effect after laundering. They absorb the UV rays of the sunlight and then fluoresce as a bright blue.

Optical Brightener Monitoring (OBM) is a new method for detecting fluorescent materials in water samples. It is based on a method used to measure the presence of strongly fluorescent tracer dyes.

Briefly, cotton pads that are free of fabric brighteners are used for checking the presence of optical brighteners in water samples. Cotton pads are soaked in the water sample and then dried in a darkened room. The pads are then viewed with ultraviolet (UV) light to check for the presence of fluorescence. This is an inexpensive, but much less sensitive, method for the detection of fluorescence compared to fluorometers.

Homemade OBM traps are inexpensive and easy to make. Table F4.1 lists the average costs of the supplies needed to make OBM traps, most of which can be found at a local hardware or home improvement store.

The following tests were conducted to determine how effective this test would be to detect inappropriate discharges originating from washwaters or sanitary wastewaters to storm drainage systems. This test may have several advantages compared to other methods used to detect these wastewaters: fluorometers are very expensive, detergent analyses can be hazardous, and the boron content of detergents varies widely. In addition, the OBM method usually involves placing the test pads in the targeted water for extended periods (up to several days) and may therefore be sensitive to intermittent discharges. These tests were therefore conducted to determine the sensitivity of the OBM method and to investigate its reliability under both field and laboratory conditions.

Table F4.1: Start-Up Costs for Optical Brightener Monitoring
(Source: Sargent and Castonguay, 1998)

Equipment	Cost
25 - 1/2” wire mesh (cages)	$ 75.75
42 feet black plastic mesh	$ 4.50
100 yards 20 lb. test monofilament	$ 2.00
500 elastics	$ 10.00
1000 staples	$ 5.00
Unexposed labels	$ 12.00
5 boxes plastic bags	$ 5.00
200 craft sticks	$ 2.00
25 aluminum spikes	$ 23.00
1 case unwashed cotton pads	$ 88.00
12 rubber gloves	$ 16.00
6 watt UV light with 2 bulbs	$ 240.00
Total	**$ 483.25**

Test Procedure

Step One:
Care should be taken so that samples are handled properly with no cross contamination. Gloves free of fabric brightener should be worn at all times when handling the test materials. The field test kit includes brightener-free cotton pads and a sampler cage to hold the pads in place if they are to be deployed for extended periods. The sampler cage is a non-metallic plastic, or a vinyl coated black wire cage having 0.5" openings. The cage consists of two hinged pieces approximately 5" by 5". This cage should be fabricated so that it will hold the fabric pads at approximately a 30 to 45 degree angle. The open end of this cage is held closed with an elastic band. A 4 to 6 watt long-wave fluorescent UV ultraviolet light is used to observe fluorescence on the fabric.

Step Two: (Placement)
At an outfall or small stream sampling location, the wire cage is secured by a heavy monofilament fishing line tied to a branch, a rock, or an aluminum spike. In sampling catchbasins, the wire cage is lowered into the catch basin by the monofilament fishing line that is then tied to the grate cover or other object. The wire cage is suspended within the water flow. The fabric pad is generally exposed for seven days. If intermittent flows are present, the device may be kept for an even longer period. However for quick sampling, the pad needs to be exposed to a water sample for at least one hour. If rust or sediment obscures the sample, then the duration needs to be shortened.

Step Three: (Retrieval)
After the samplers are retrieved from the water, the pads are removed from the sampling device. The pads are then rinsed in the sampling water to remove any surface sediment, and squeezed to remove excess water without tearing or ripping the pads. The pads are also labeled (see Figure F4.2).

All labels must be analyzed using the UV light to check for the presence of brighteners, as most white paper contains optical brighteners that can interfere with the optical brightener measurements of the pads. Label information should include, location, day/time of placement, and day/time of removal. The stiff paper labels are stapled to the retrieved sampling pads, placed in a zip lock bag, and kept in the dark as they are being transported to the laboratory. Upon arrival at the laboratory, the pads are dried in a darkened room (where they will not come into contact with direct sunlight) by hanging on a non-cotton monofilament line (see Figure F4.2). The line should either be replaced or cleaned by a cotton pad after every use.

Step Four: (Analysis)
The pads are viewed in a darkened room using a long-wavelength UV light source. The pads are easiest to examine in a dark room using a special UV lamp viewing cabinet. A non-exposed pad is used as a control. The pad will fluoresce if it is positive for brighteners, while it will be noticeably drab like the control pad if it is negative. Uneven exposure of the pad to optical brighteners may result in uneven fluorescence of the pad. If the reason for partial fluorescence can be explained then the pad should be regarded as positive. Specks or spots of fluorescence on the pads may be ignored.

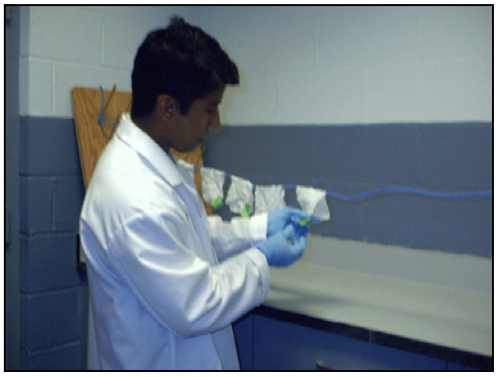

Figure F4.2: Labeling the Pad

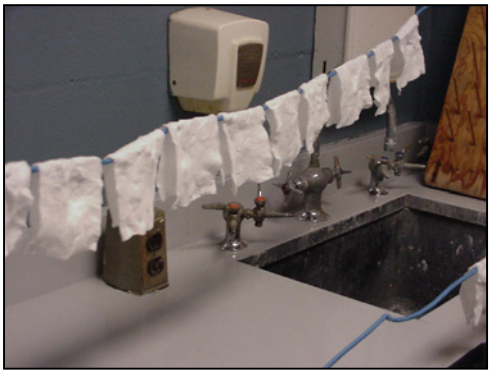

Figure F4.3: Drying the Pads

Method Modifications

While reviewing the prior methods for the OBM for inappropriate discharge detection, the following issues were brought up:

a) Do the pads need to be left in the field for extended periods and how long should the pads be exposed to the sample water?

b) Are there any detrimental effects of direct exposure to sunlight while drying the cotton pads?

c) What is the sensitivity of the OBM compared to the other tests used to detect washwaters and sanitary wastewaters?

The above points are discussed in the following paragraphs.

Leaving the cotton pad and the sampling device at the sampling location

If there is continuous flow at an outfall, there is no need to keep the pads at the outfall for extended periods. If grab samples are collected from the flowing outfalls for later chemical tests, a separate sample bottle can be conveniently collected for optical brightener tests. During our analyses, the cotton pads were immersed in the sample bottles at the time of sample collection. This sampling modification greatly reduced the time and effort needed to conduct the tests. Our initial tests indicated that the high sediment loads associated with the outfall discharges would hinder the ability to measure the fluorescence due to coating the fabrics with silt. If the pads were placed in the OBM sample bottles when the water was collected, the time required to bring the samples to the laboratory was thought to be sufficient to affect the pads. Tests were conducted in the laboratory to determine the time needed to affect the pads. The standard procedure used at least a one hour exposure period.

Direct exposure to sunlight while drying the cotton pads.

There was a concern related to the degradation of fabric fluorescence in the presence of sunlight, especially after the fluorometer tests indicated significant decreases in water sample fluorescence during the first hour or two after detergent mixing. In order to test this concern, two samples were prepared with the same concentration of detergents. Two cotton pads were immersed in each of the bottles. One was dried under the direct exposure of sunlight, while the other one was dried in a dark room. After 24 hours, both sets of pads gave the same fluorescence under the ultraviolet light. Therefore, it was concluded that direct sunlight exposure to the dried cotton pads did not affect the test results.

Other sampling and laboratory practices that were important included using gloves while handling the pads, and testing the cotton pads for fluorescence under the UV lamp before their use.

Laboratory Verification using Standard Samples and Field Use in Cribbs Mill Creek

The basic OBM method is a presence/absence test, with unknown sensitivity. In order to make this test more useful, additional tests were conducted. The initial test used different Tide detergent standards. Tide detergent samples were made with concentrations of 0.5 mg/L, 5 mg/L, 10 mg/L, 20 mg/L, 30 mg/L 50 mg/L, 100 mg/L, and 500 mg/L. Samples from each dried test pad were attached onto a card, as shown in Figure F4.4.

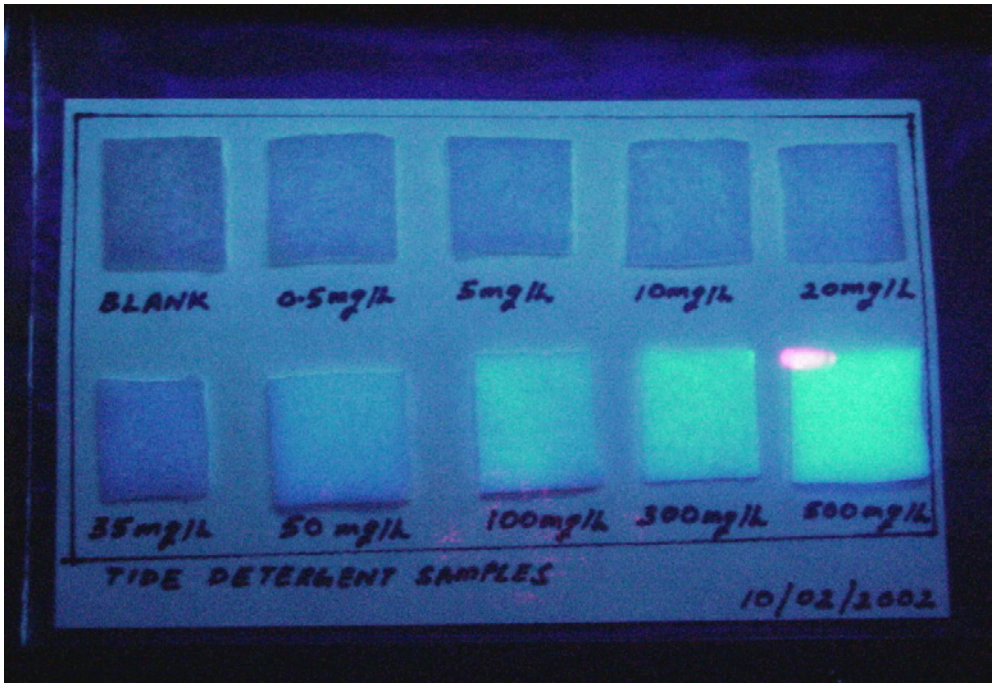

Figure F4.4: Standard Tide OBM Pads

As can be seen in Figure F3.4, concentrations below 35 mg/L all look identical. The 50 mg/L Tide solution (the first one with an obvious fluorescence response) is representative of a full-strength washwater as typically used in household laundry. Thus, it may be concluded that the OBM method may not be useful for samples having anything less than full-strength washwaters.

The maximum fluorescence concentration obtained from the Cribbs Mill Creek samples was 17mg/L (as Tide), and no positive responses for fluorescence using the OBM method were found.

Conclusion

This test was originally designed to identify faulty septic systems and storm drainage systems using fluorescent dyes. The fluorescent dyes (Fluorescence and Rhodamine FWT) used in these types of tests are very strong dyes and are used in moderate concentrations. They are therefore much easier to be detected by the cotton pads and the OBM method than the fabric brighteners in washwaters. OBM is a quick, easy, and inexpensive method, but can only reliably detect undiluted washwaters, and likely will miss the more common diluted washwaters found as inappropriate discharges. Other simple methods exist that are more sensitive, although the OBM method may be most suitable if intermittent discharges of undiluted washwaters are expected.

Appendix F5. IN-HOUSE ANALYTICAL CONSIDERATIONS FOR INDICATOR PARAMETERS

Introduction

Program managers need to understand the basic analytical options and safety considerations, for each analytical method used to measure indicator parameters. This understanding helps program managers choose what indicator parameters to collect and where they should be analyzed. This section provides a summary of the basics.

Table F5.1 summarizes the recommended analysis method associated with each indicator parameter. An extended description of each analysis method is provided below.

Colorimetric – Colorimetric methods utilize specialized instruments such as a colorimeter or a spectrophotometer (Figure F5.1). The two instruments are similar and quantify parameter concentrations by adding reagents to the sample and passing through a defined spectrum of light. In general, spectrophotometers can analyze a much broader range of parameters than colorimeters.

Table F5.1: Analytical Considerations for Illicit Discharge Indicator Parameters

Indicator Parameter	Method	Analysis Type	Limit of Detection
Ammonia	*HACH Method 8155*	Colorimetric	0.01 mg/L
Boron	*HACH Method 10061*	Colorimetric	0.02 mg/L
Chlorine	*HACH Method 8021*	Colorimetric	0.02 mg/L
Color	*HACH Color Wheel*	Color Comparator	1 color unit
Conductivity	Various Probe or Meter Techniques	Probe or Meter	N/A
Detergents – Surfactants	*Chemetrics Chemets*	Color Comparator	0.25 mg/L
E. coli, Total Coliform, Enterococci	IDEXX: Colilert Or Enterolert	IDEXX: Colilert Or Enterolert	1 MPN/100 mL
Fluoride	*HACH Method 8029*	Colorimetric	0.01 mg/L
Hardness	*HACH Method 8213*	Titration	1 mg/L
Potassium	*HACH Method 8049*	Colorimetric	0.1 mg/L
	Horiba Probe	Probe	5 mg/L
PH	Probe (Various)	Probe or Meter	1 pH unit
Turbidity	Various Turbidity Meters	Probe or Meter	1 NTU

Figure F5.1: Spectrophotometer

Color Comparator – This analysis method is a less quantitative version of the colorimetric method. Samples are prepared by adding reagents, and assessing the color in comparison to a color cube (see Figure F5.2) or color disk that assigns a concentration for different color shades.

Figure F5.2: HACH Color Cube Comparator

Probes – These methods use a probe to pass an electrical current through the sample for specific light wavelength (for most indicators) or measure the scatter of light (for turbidity). While results are immediate, lab analysts need to frequently calibrate the probe using standard solutions to assure accurate data.

Titration – Titration techniques measure the concentration of indicator parameters by determining the amount of a reagent needed to produce a specific reaction in the sample, which is often indicated by a color change. Lab analysts carefully record the amount of reagent added to the sample using a "burette," which is a graduated cylinder with a valve-controlled opening at the bottom. An alternative and more precise technique is a digital titrator. Both methods rely on equations or lookup tables that relate to the amount of reagent added to the estimated concentration of the indicator parameter.

IDEXX Techniques: Colilert or Colisure - These proprietary methods are used to measure *E. coli*, total coliform and Enterococci bacteria. Samples are sealed along with a reagent in a specialized tray that is then placed into an incubator for 24 hours. The analyst then measures the number of cells in the tray that have changed color or shine under a fluorescent bulb, which is used to indicate the amount of bacteria in the sample (Figure F5.3). The IDEXX method uses a standard chart to relate the number of cells that have a positive reaction to the presence of bacteria. The IDEXX method is fairly simple and safe, but requires fairly expensive equipment.

Safety and Waste Management Considerations

Each analysis method has special safety and waste disposal considerations, which are outlined in Table F5.2.

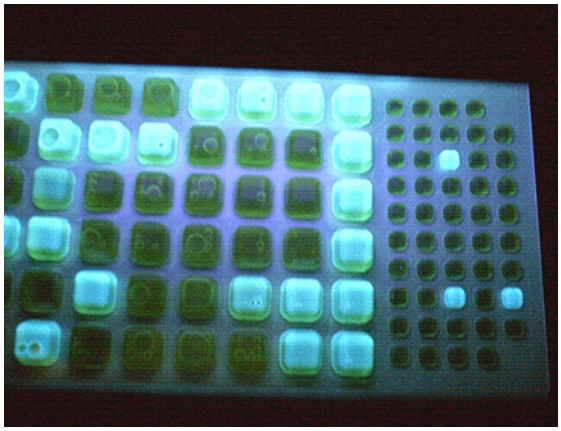

Figure F5.3: IDEXX Results

Table F5.2: Special Safety and Waste Management Considerations			
Indicator Parameter	**Method**	**Major Health Risks**	**Special Disposal Requirements**
Detergents – Surfactants	Chemetrics Chemets	Carcinogenic. Causes dermatitis and lung infection. Need to provide ventilation.	Hazardous Waste
E. coli; Total Coliform; Enterococci	*IDEXX: Colilert Or Enterolert*	OK	Potential Biohazard (Consult State Health Agency for requirements)
Fluoride	HACH Method 8029	Causes erosion of teeth.	Reagent is a hazardous waste.
Hardness	HACH Method 8213	No major	Reaction produces a hazardous waste.

TIP

The IDEXX technique requires a special adaptation when used to measure *E. coli* in discharges from storm drain outfalls. The concentration that distinguishes sewage from other dischares is greater than 12,000MPN/100ml. Using this method, the maximum redable concentration is only 2,619MPN/ml. Dilute outfall samples to 10-20% of their original concentrations with deionized water in order to read the very high concentrations of *E. coli* that identify sewage discharges.

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